AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Evaluation of Multiple Methods for Detection of Gastrointestinal Colonization of Carbapenem-Resistant Organisms from Rectal Swabs

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Rectal swabs from high-risk patients were screened for carbapenem-resistant organisms (CROs) using several methods. The direct MacConkey plate method was the most sensitive for CROs (95%), while chromID CARBA and the Check-Direct CPE screen assay were the most sensitive for the detection of carbapenemase-producing organisms (CPOs) (100%; all bla_{KPC}). All methods had a specificity of >90% for CROs, and for CPOs, the specificity ranged from 85 to 98%. Broth enrichment methods performed poorly compared to direct inoculation methods, negating the need for the broth enrichment step.

In 2013, the U.S. Centers for Disease Control and Prevention (CDC) assigned the highest threat level to carbapenem-resistant *Enterobacteriaceae* (CRE). Additionally, the CDC designated multidrug-resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter baumannii* as serious threats because they are resistant to nearly all available antibiotics, including carbapenems—declaring that they require urgent public health attention (1). Optimal screening methods for rapid detection of carbapenem-resistant organisms (CROs) have yet to be established (2). Currently described methods include broth enrichment, direct selective culture, chromogenic media, and detection of carbapenemase genes directly from rectal swabs (3–9). The objectives of this study were to (i) evaluate multiple methods for screening CROs from rectal swabs and (ii) to determine the prevalence of gastrointestinal colonization with CROs among high-risk inpatient populations.

Two-hundred thirteen remnant vancomycin-resistant enterococcus (VRE) surveillance rectal ESwabs (Copan, Murrieta, CA) were collected in a non-outbreak setting from four distinct inpatient populations at The Johns Hopkins Hospital, Baltimore, MD. ESwabs were collected upon hospital unit admission and weekly thereafter until unit discharge. Consecutive rectal ESwabs were collected from medical and surgical intensive care units (MICUs and SICUs, respectively), an oncology ward, and an organ transplant ward over a 2-week period. The ESwabs were vortexed, and the remnant liquid Amies broth was aliquoted to cryovials and frozen at −70°C until further testing was performed. From each ESwab broth, five different methods for the detection of CRO were set up in parallel. The five methods included (i) the CDC broth enrichment method with ertapenem for selection (3), (ii) a modified CDC broth enrichment method using ertapenem and vancomycin for selection (3), (iii) a direct MacConkey plate with ertapenem disks (4, 6), (iv) a chromogenic chromID CARBA agar plate method (with the new reformulated medium; package insert version 20157 A-en-2013/02, reference no. 414012 [bioMérieux, Marcy l'Étoile, France]), and (v) the Check-Direct CPE screen assay for the BD MAX instrument (Check-Points, Wageningen, The Netherlands; Becton Dickinson, Sparks, MD). The ESwab broth was first vortexed for 5 s, 100 μl of ESwab broth was inoculated into each of the medium types, and 25 μl was inoculated into a DNA sample buffer tube (SBT) for the Check-Direct CPE assay. All methods were performed as previously described, with a few exceptions as described below. For the modified CDC broth enrichment method, a vancomycin disk was added to the broth to inhibit potential Gram-positive organisms from breaking through the ertapenem disk, resulting in false-positive turbid broths. For both CDC broth enrichment methods (with and without vancomycin), an ertapenem disk was added to the MacConkey plate on subculture from turbid broths. Any isolate that grew within 27 mm of the ertapenem disk was further identified, and antimicrobial susceptibility testing (AST) was performed (6). Finally, the Check-Direct CPE assay was performed as per the manufacturer's instructions on the BD MAX instrument, with the exception of the sample volume setting, which was set to 937.5 μl. The run was validated when the positive and negative control threshold cycle (C_T) values were within the acceptable limits with appropriate amplification curves. Specimens were determined to be positive if a C_T value was provided for one of the targets and for the sample processing control (SPC [internal control]).

All organisms that met the criteria for further evaluation (recovery of Gram-negative bacilli that grew within 27 mm of the ertapenem disk or growth on chromID CARBA medium) were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Inc., Billerica, MA), and/or identification and AST were performed with the BD Phoenix automated instrument (Becton Dickinson, Sparks, MD). AST results were interpreted following Clinical and Laboratory Standards Institute guidelines (10). *Enterobacteriaceae* resistant to one of the carbapenems tested (ertapenem and meropenem) or glucose-nonfermenting Gram-negative bacilli resistant to meropenem were further evaluated by the

Received 11 March 2016 **Returned for modification** 27 March 2016
Accepted 31 March 2016

Accepted manuscript posted online 6 April 2016

Citation Simner PJ, Martin I, Opene B, Tamma PD, Carroll KC, Milstone AM. 2016. Evaluation of multiple methods for detection of gastrointestinal colonization of carbapenem-resistant organisms from rectal swabs. J Clin Microbiol 54:1664–1667. doi:10.1128/JCM.00548-16.

Editor: C.-A. D. Burnham, Washington University School of Medicine

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Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.00548-16.

TABLE 1 Summary of method comparison results for detection of carbapenem-resistant organisms from rectal swabs

Parameter	No. (%) by[a]: CDC	CDC + Vanco	Direct MAC	chromID CARBA	Check-Direct CPE screen	Total
Broth enrichment						
Cultures with turbid broths	157 (73.7)	72 (33.8)	NA	NA	NA	NA
Cultures with growth on subculture from turbid broth to MacConkey plate with ertapenem disk	71 (33.3)	35 (16.4)	NA	NA	NA	NA
Direct selective culture						
Cultures with any growth on plates after inoculation with specimen	NA	NA	146 (68.5)	29 (13.6)	NA	NA
Recovered organisms by culture-based methods						
Cultures positive with GNB recovered that required further workup[b]	27 (12.7)	21 (9.9)	36 (16.9)	26 (12.2)	NA	41 (19.2)
Cultures positive with CROs	11 (5.2)	8 (3.8)	18 (8.5)	15 (7.0)	NA	20 (9.4)
Cultures positive with CPOs	2 (0.9)	1 (0.5)	4 (1.9)	5 (2.3)	NA	5 (2.3)
Check-Direct CPE screen molecular results						
Swabs carbapenemase gene positive	NA	NA	NA	NA	10 (4.7)	10 (4.7)

[a] See Table S1 in the supplemental material for expanded Table 1 results summarizing the organisms recovered. CDC, CDC broth enrichment method; CDC + vanco, CDC broth enrichment method with the addition of a vancomycin disk; Direct MAC, direct MacConkey plate with ertapenem disks; NA, not applicable.
[b] Criteria for further workup included recovery of Gram-negative bacilli (GNB) that grew within 27 mm of the ertapenem disk for the CDC, CDC + Vanco, and Direct MAC methods or growth on chromID CARBA medium.

Carba NP assay to identify carbapenemase production (10). If an isolate was determined to be positive by the Carba NP, the molecular genotype was determined by the Check-MDR CT103XL assay as previously described (Check-Points, Wageningen, The Netherlands) (11). Two ATCC strains (*Klebsiella pneumoniae* carbapenemase [KPC]-producing strains ATCC BAA-1705 and ATCC BAA-1706) and a set of 10 positive controls producing a known and diverse range of carbapenemases (bla_{KPC}, bla_{NDM}, bla_{OXA-48}-like, or bla_{VIM}) were inoculated (0.5 McFarland standard of pure cultures) into each of the five study arms to assess performance. Statistical analysis was performed using GraphPad QuickCalcs (GraphPad Software, La Jolla, CA).

The 213 rectal swabs were collected from 191 unique patients; with 123 (57.7%) collected upon ward admission and 90 (42.3%) collected on follow-up surveillance cultures during the ward stay. Of the 213 swabs, 69 (32.4%), 58 (27.2%), 57 (26.8%), 8 (3.8%), and 21 (9.9%) were collected from a transplant ward, MICU, SICU, an oncology ward, and a variety of other wards, respectively. The overall prevalences of colonization among the evaluated patient populations studied were 9.4% ($n = 20$) for CROs and 2.3% ($n = 5$) for carbapenemase-producing organisms (CPOs). The respective prevalences of CRO and CPO colonization were as follows: transplant ward, 11.5% ($n = 8$) and 5.8% ($n = 4$); MICU, 8.6% ($n = 5$) and 1.7% ($n = 1$); SICU, 8.7% ($n = 5$) and 0%; oncology ward, 12.5% ($n = 1$) and 0%; and other units, 4.7% ($n = 1$) and 0%.

A comprehensive method evaluation study was completed to determine the most sensitive and specific test for the detection of CROs and CPOs from rectal swabs. The results of the method comparison study are summarized in Table 1. Overall, 20 CROs, including 5 CPOs, were recovered by 1 of the 4 different culture-based methods. The non-carbapenemase-producing CROs ($n = 15$) included 7 *Stenotrophomonas maltophilia* (46.7%), 4 *Enterobacter cloacae* (26.7%), 2 *Pseudomonas aeruginosa* (13.3%), and 1 each of *Escherichia coli* and *Hafnia alvei* (6.7%). All five CPOs recovered were KPC producers and included 2 *E. cloacae* (40%), 2 *Citrobacter amalonaticus* (40%), and 1 *Klebsiella pneumoniae* (10%). The Check-Direct CPE screen was positive for a carbapenemase gene in 10 (4.7%) of the rectal swabs. The 10 positive swabs included 7 bla_{KPC}, 2 bla_{VIM}, and 1 $bla_{OXA-48-like}$. The CPOs for 5 of the 10 positives were recovered by one to multiple culture-based methods. No growth was observed by any of the culture-based methods for 2 bla_{VIM} (cycle threshold [C_T], 39.7 and 40.8), 2 bla_{KPC} (C_T, 29.6 and 40.5), and 1 $bla_{OXA-48-like}$ (C_T, 40.6). The two ATCC strains and 10 positive controls were appropriately recovered by each of the culture methods and detected by the Check-Direct CPE screen assay. The performance characteristics of each method are summarized in Table 2. The "gold standard" for comparison was the cumulative results recovered from any culture-based methods (i.e., 20 CROs and 5 CPOs total).

The direct MacConkey plate method with ertapenem disks was the most sensitive for CRO detection (95%), while the chromID CARBA medium (100%; $n = 5$; all bla_{KPC}) was the most sensitive for CPO detection among culture-based methods. All culture-based methods had a specificity of >90% for CRO detection, which decreased to 84.6 to 90.4% for the detection of CPOs. We noted that for all of the direct MacConkey plates with ertapenem disks, the ertapenem disk in the first quadrant of growth was sufficient for the measurement of the zone of inhibition. Thus, we elected to implement into clinical practice the direct MacConkey plate method with the addition of ertapenem to the first quadrant and a meropenem disk to the second quadrant (as opposed to a second ertapenem disk) to enhance recovery of CROs/CPOs if a meropenem-susceptible nonfermenter were also to grow. The chromID CARBA medium is currently available in the United

TABLE 2 Performance characteristics of the various methods to detect carbapenem-resistant organisms and KPC-producing organisms from rectal swabs

Performance characteristic[a]	% (95% CI) by[b]:				
	CDC	CDC + Vanco	Direct MAC	chromID CARBA	Check-Direct CPE screen
Sensitivity					
CROs	55.0 (32.0–76.2)	40.0 (20.0–63.6)	95.0 (73.1–99.7)	75.0 (50.6–90.4)	
CPOs	40.0 (7.3–83.0)	20.0 (1.1–70.1)	80.0 (29.9–98.9)	100 (46.3–100)	100 (46.3–100)
Specificity					
CROs	91.7 (86.7–95.0)	93.3 (88.5–96.2)	91.2 (86.0–94.6)	94.3 (89.8–97.0)	
CPOs	88.0 (82.3–91.9)	90.4 (85.3–93.8)	84.6 (78.8–89.1)	89.9 (84.8–93.5)	97.6 (94.2–99.1)
PPV					
CROs	40.7 (23.0–61.0)	38.1 (19.0–61.3)	52.7 (35.7–69.2)	57.7 (37.2–76.0)	
CPOs	7.4 (1.3–25.8)	4.8 (0.02–25.9)	11.1 (3.6–27.0)	19.2 (7.3–40.0)	50.0 (20.1–79.9)
NPV					
CROs	95.2 (90.7–97.6)	93.8 (89.1–96.6)	99.4 (96.4–100)	97.3 (93.5–99.0)	
CPOs	98.9 (95.0–99.6)	97.9 (94.4–99.3)	99.4 (96.4–100)	100 (97.5–100)	100 (97.7–100)

[a] The "gold standard" for comparison was the cumulative results recovered across the various culture-based methods (i.e., 20 carbapenem-resistant organisms [CROs] and 5 KPC-producing carbapenemase-producing organisms [CPOs] total).
[b] 95% CI, 95% confidence interval; CDC, CDC broth enrichment method; CDC + Vanco, CDC broth enrichment method with the addition of a vancomycin disk; Direct MAC, direct MacConkey plate with ertapenem disks.

States as a research-use-only product but will be undergoing a clinical trial to obtain FDA clearance in the near future.

The CDC broth enrichment methods (±vancomycin) performed poorly compared to direct inoculation of selective culture methods. All false-negative cultures were overgrown with meropenem-susceptible *P. aeruginosa*, which may have masked the recovery of the CROs/CPOs. The use of meropenem as the selective agent may have prevented the overgrowth of meropenem-susceptible *P. aeruginosa* and allowed the recovery of certain CROs/CPOs. However, this may have also resulted in suppression of CRE or carbapenemase-producing *Enterobacteriaceae* isolates that were ertapenem resistant but meropenem susceptible (i.e., 8 CRE isolates recovered in this study were ertapenem resistant but meropenem susceptible, including the 2 KPC-producing *E. cloacae* isolates). In addition, the vancomycin disk added to the broth enrichment step in the modified CDC arm resulted in fewer turbid broths (33.8% versus 73.7%; $P < 0.0001$). However, surprisingly the addition of vancomycin also hindered the ability to recover Gram-negative organisms and performed worse than the CDC broth enrichment for the detection of CROs/CPOs (see Table S1 in the supplemental material). Overall, the broth enrichment methods performed poorly compared to direct selective culture methods that lacked broth enrichment. Similar to our findings, other method comparison studies have noted that broth enrichment resulted in poorer results than direct selective culture plating methods (7, 8, 12). However, a recent study demonstrated that broth enrichment prior to inoculation to chromID CARBA medium resulted in an increased sensitivity compared to a direct inoculation method (13). These varied results may be due to differences in the carbapenem utilized for selection (ertapenem versus meropenem) in the broth enrichment step, differences in patient populations where *P. aeruginosa* colonization is less frequent, and differences in circulating CROs/CPOs.

The Check-Direct CPE screen assay demonstrated excellent sensitivity (100%, $n = 5$, all bla_{KPC}) and specificity (97.6%) for the detection of carbapenemase genes. However, positive results with high threshold cycle values (most had a C_T of >39) resulted in a decreased positive predictive value (PPV) of 50% and a negative predictive value (NPV) of 100%, in comparison to culture-based methods. These positives may reflect low-level colonization below the limit of detection of culture, or they may represent false-positive results due to off-target amplification of closely related sequences found among gastrointestinal flora (5). However, even if these low-level positive results reflect colonization, the clinical significance and likelihood of transmission in low-level carriers are unknown. This is the first study evaluating the Check-Direct CPE screen assay on the BD MAX instrument. Other studies published on the Check-Direct assay were performed on the NucliSENS easyMag instrument and reported similar results with high sensitivity and lower specificity than culture-based methods (5, 8, 14). Although molecular methods provide a more rapid result (~3 h from sample receipt) than culture-based methods (24 to 48 h), their major pitfall is the overall cost per test (~$50). Thus, the role of molecular assays in screening for CPOs will likely be the most impactful in an outbreak setting.

Limitations of this study include (i) the detection of CPOs based on carbapenem resistance with reflex to the Carba NP assay (i.e., some organisms harboring carbapenemases may have been missed if they had MICs below the resistance breakpoint or were not detected by the Carba NP assay [i.e., OXA-48-like producers]) and (ii) the lack of recovery of other carbapenemase types in the clinical cohort. Only KPC producers were recovered in this study.

This method comparison found that the direct MacConkey plate method with ertapenem disks was the most sensitive test for CRO detection, while the chromID CARBA and Check-Direct CPE screen methods were the most sensitive for the detection of bla_{KPC}-positive carbapenemase producers (100%). In this study, broth enrichment methods (±vancomycin) performed poorly compared to direct inoculation of selective culture methods, negating the need for the broth enrichment step. We believe the aforementioned method comparisons should be repeated with a larger, diverse sample of isolates.

ACKNOWLEDGMENTS

We thank Tsigereda Tekle, Jo-Anne Miller, Tracy Howard, and Ani Voskertchian for invaluable help with this project, Check-Points for providing 6 Check-Direct CPE screen kits, and bioMérieux for providing 100 sets of the chromID CARBA media.

FUNDING INFORMATION

This study was funded in part by the Centers for Diseases Control and Prevention's Prevention Epicenters Program through collaborative agreement number 1 U54 CK000447-001 and by the Fisher Center Discovery Program award supported by the Sherrilyn and Ken Fisher Center for Environmental Infectious Diseases, Division of Infectious Diseases of the Johns Hopkins University School of Medicine. The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention, the Department of Health and Human Services, or the Sherrilyn and Ken Fisher Center for Environmental Infectious Diseases. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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